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RESEARCH ARTICLE

Synthesis of 2-hydroxybenzohydrazide derivatives with microwave irradiation and activity against *Escherichia coli*

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Abstract

Background: This study synthesised derivatives of 2-hydroxybenzohydrazide using microwave irradiation (green chemistry) and tested their antibacterial activity against *Escherichia coli*. **Objective:** The reaction yielded N'-Benzylidene-2-hydroxybenzohydrazide, N'-(2-Methoxybenzylidene)-2-hydroxybenzohydrazide, and N'-(4-Methoxybenzylidene)-2-hydroxybenzohydrazide compounds. Prior to compound synthesis, docking was performed with the ENR inhibitor receptor (Pdb.1C14), to determine the antibacterial activity test. **Method**: The synthesis was carried out using a condensation reaction. Identification of compounds was carried out by UV-VIS, FTIR, and 1H-NMR spectroscopies. Antibacterial activity against E.*coli* was evaluated using the well method. Molecule docking study was performed using Mollegro virtual docker. **Results:** The percentage yield of the synthesis of 2-hydroxybenzohydrazide derivatives was obtained at 68-81%. Docking with the ENR inhibitor receptor for these compounds resulted in a lower rerank score than the initial compound (methyl salicylate). These compounds exhibited antibacterial activity against E.*coli*. **Conclusion:** These compounds can synthesised using microwave irradiation. N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide has greater activity against E.*coli.*

Introduction

Drug development is important in the pharmaceutical industry. The use of conventional methods for the development of drug compounds through organic reactions that require high heat has been widely used. In addition, a hot surface inside the flask can cause localised overheating, which results in the decomposition of products, substrates, and other reagents when heating is applied for a long time. Therefore, it is necessary to find another reaction method that produces a synthesis reaction with rapid filtration, a fairly high reaction product, and the expected quality. Synthesis reaction with microwave irradiation is one of the new techniques in the development of new chemical compounds into drugs (Kappe, 2019; Stadler & Kremsner, 2014).

Hydrazide derivatives are organic compounds characterised by the presence of the –NH-N=CH- group in their molecule. Some widely used antibacterial drugs, such as furacilin, furazolidin, and ftivazide are known to contain this group (Ozkay *et al*., 2010). The condensation of carbonyl-containing compounds with primary amines produces Schiff base compounds bearing C=N double bonds, which is an essential structural requirement for biological activities, especially for antibacterial, antimicrobial, antifungal, and antitumor activities (Raparti *et al.,* 2009; Wang *et al.,* 2013). The synthesis can be performed by using the

conventional method and microwave irradiation, but this synthesis will be performed by the microwave method in a shorter time. Results obtained from this method are more environmentally friendly because it does not use solvents. Reduced use of toxic solvents and energy savings by campaign green chemistry (Kabalka, Wang & Pagni, 2001a; Paul, Nanda & Gupta, 2003a). Molecular docking of 2-hydroxybenzohydrazide derivatives (N'-benzylidene-2-hydroxybenzohydrazide, N'-(2-methoxybenzylidene)-2-hydroxybenzo hydrazide and N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide) was carried out using the MVD (Mollegro Virtual Docking) method. The receptor used ENR inhibitors (Pdb.1C14). The study also includes antibacterial activity against *Escherichia coli* of the compounds. Based on the background above, the problem is formulated in this study: whether microwave irradiation can be used for the synthesis of 2-hydroxybenzohydrazide and its derivatives? The benefits of research can inform new technology about the method of condensation reaction through the application of microwave irradiation to synthesise 2-hydroxybenzohydrazide compounds and their derivatives (McMurry, 2008; Habii & Marvi, 2006). The reactions are described in the following Figures 1 and 2.

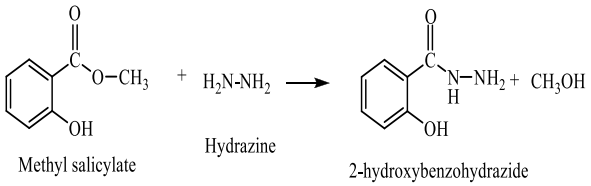

Figure 1: The first step of the reaction is the formation of an intermediate compound 2-hydroxybenzohydrazide

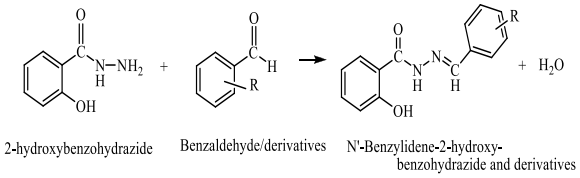

Figure 2: The second step of the reaction is the formation of *N*'-Benzylidene-2-hydroxybenzohydrazide derivatives

Methods

Materials

All chemicals were used in this study from commercial sources. Methyl salicylate, hydrazine hydrate, benzaldehyde, 2-methoxybenzaldehyde, 4-methoxybenzaldehyde, ethanol 96%, chloroform, ethyl acetate, acetone, hexane, KBr, and silica gel 60 GF254 were purchased from Merck.

Instrumentation

Glassware is commonly used in chemical synthesis laboratories. Sanyo microwave EM-S 800 Watt, UV-Vis Spectrophotometer HEWLETT PACKARD 8452A, Spectrophotometer Buck Scientific IR M-500, FT-NMR spectrometer JEOL ECS-400.

Preparation of 2-hydroxybenzohydrazide with microwave irradiation (Jain et al., 2007)

An equimolar mixture of methyl salicylate (10 mmol) and hydrazine hydrate (20 mmol) entered the Erlenmeyer flask (25 ml) and was stirred until homogeneous. The mixture was subjected to microwave irradiation for 8 minutes while stirring every 2 minutes (160 W). The progress of the reaction was monitored by TLC. The mixture was cooled to room temperature and then added to 20-30 ml of distilled water, filtered, and washed with ethanol. Crystals were obtained by recrystallisation with absolute ethanol, the purity test chromatography melting point, and thin layers using three different eluents. Identification was performed using UV-VIS, Infrared and 1H-NMR spectroscopies.

Preparation of 2-hydroxybezohydrazide derivatives by microwave-irradiation (Hayes & Brittany, 2002; Jain et al., 2007)

An equimolar mixture of 2-hydroxybenzohydrazide (10 mmol), benzaldehyde/derivatives (20 mmol), and 12 ml ethanol were stirred until homogeneous. Ethanol was evaporated until exhausted. The mixture was subjected to microwave irradiation for 8 minutes while stirring every 2 minutes (160 W/320 W). The progress of the reaction was monitored by TLC. The mixture was cooled to room temperature and then added to 20-30 ml of distilled water, filtered, and washed with ethanol. Crystals were obtained by recrystallisation by absolute ethanol, the purity test chromatography melting point, and thin layers using three different eluents. Identification was performed using UV-VIS, Infrared and 1H-NMR spectroscopies.

Antibacterial test

The compounds were tested for in vitro antibacterial activities against *Escherichia coli* using the paper disc diffusion method (Balouri, Sadiki & Ibnsouda, 2016). Amoxicillin was used as the reference drug.

Results

Synthesis of 2-hydroxybenzohydrazide derivatives by condensation reaction carried in two reaction steps. The first step reacting methyl salicylate and hydrazine hydrate to obtain 2-hydroxybenzohydrazide compounds with 160 Watt microwave irradiation for 2-8 minutes. The second step to get 2-hydroxybenzohydrazide derivatives (N'-benzylidene-2-hydroxybenzohydrazide, N'-(2-methoxybenzylidene)-2-hydroxybenzohydrazide, and N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide). This method was chosen because it is a practical path and does not use toxic solvents. In addition, the reaction time used for the synthesis of compounds 2-hydroxybenzohydrazide derivatives is relatively short at less than 10 minutes, and the reaction is carried out in the absence of toxic solvents. So this method will fit the green chemistry term with reduced use of toxic solvents and energy saving (Kabalka, Wang & Pagni, 2001b; Paul, Nanda & Gupta, 2003b). 2-Hydroxybenzohydrazide derivatives were synthesised by reacting 2-hydroxybenzohydrazide with benzaldehyde or benzaldehyde derivatives (4-methoxybenzaldehyde and 2-methoxybenzaldehyde) in ethanol. Ethanol was used as the solvent, evaporating to run out (Kabalka, Wang & Pagni, 2001c).

Characterisation of 2-hydroxybenzohydrazide

Obtained in 86% yield as a crystalline form of white needles. Chromatography purity test results with thin-layer show a stain, m.p. 140-141°C. UV-Vis (Et-OH), nm: 210, 236 and 300. FT-IR (KBr in cm-1): 1645 (-C=O amide), 3266 (-OH phenolic), 1590 (-C=C- aromatic), 1532 (-NH), 1354 (C-N), 3053 (Csp2-H), 750 (orto substitution on benzena), 3266 and 3319 (-NH2). 1H-NMR (400 MHz, DMSO-d6, ppm): 7.36 (t, J=10,8 Hz,1H, C6H4-), 7.76 (d, J=6,3 Hz, 1H, C6H4-), 6.93-6.75 (m, 3H, C6H4-) (Silverstein *et al.,* 2005d; Pavia *et al.,* 2009a).

Characterisation of N'-benzylidene-2-hydroxybenzo-hydrazide

Obtained in 81% yield as a crystalline form of pale yellow needles. Purity test results with thin-layer chromatography show a stain, m.p. 241-242°C. UV-Vis (Et-OH), nm: 210, 302 and 314. FT-IR (KBr in cm-1): 1658 (-C=O amide), 3239 (-OH phenolic), 1613 (-C=C- aromatic), 1562(-NH), 1379 (C-N), 3027 (Csp2-H), 749 (orto substitution on benzena), 1629 (C=N), 750 (orto substitution on benzena), 3266 and 3319 (-NH2). 1H-NMR (400 MHz, DMSO-d6, ppm): 11.85 (s, 1H, -OH), 8.47 (s, 1H, -NH), 8.07-6.85 (m, 9H, two aromatic rings) (Silverstein *et al.,* 2005; Pavia *et al.,* 2009).

Characterisation of N'-(2-methoxybenzylidene)-2-hydroxybenzohydrazide

Obtained in 68% yield as a crystalline form of pale yellow needles, Purity test results with thin-layer chromatography show a stain, m.p. 241-242°C. UV-Vis (Et-OH), nm: 212 and 332. FT-IR (KBr in cm-1): 1638 (-C=O amide), 3469 (-OH phenolic), 1602 (-C=C- aromatic), 1551(-NH), 1359 (C-N), 3024 (Csp2-H), 758 (orto substitution on benzena), 1698 (C=N), 1252 (phenylalkyl eter). 1H-NMR (400 MHz, DMSO-d6, ppm): 11.82 (s, 1H, -OH), 11.98 (s, 1H,-NH), 8.78 (s, 1H, -HC=N), 7.86-6.91 (m, 8H, two aromatic rings), 3.85 (s, 3H, -OCH3) (Silverstein *et al.,* 2005c; Pavia et al., 2009c)

Characterisation of N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide

Obtained in 76% yield as a crystalline form of pale yellow needles. Purity test results with thin-layer chromatography show a stain, m.p. 222-223°C. UV-Vis (Et-OH), nm: 208 and 324. FT-IR (KBr in cm-1): 1654 (-C=O amide), 3455 (-OH phenolic), 1607 (-C=C- aromatic), 3258 (-NH), 1379 (C-N), 3070 (Csp2-H), 751 (orto substitution on benzena), 1628 (C=N), 1256 (phenylalkil eter), 2930 (Csp3-H). 1H-NMR (400 MHz, DMSO-d6, ppm): 11.90 (s, 1H, -OH), 8.29 (s, 1H, -NH), 7.90-6.50 (m, 8H, two aromatic ring), 3.80 (s, 3H, –OCH3) (Silverstein *et al.,* 2005d; Pavia *et al.,* 2009d). A docking molecule study showed that the active crystal structure of the receptor interacted with ligand compounds. Ligand-protein binding energy is represented on the rerank score of the compounds (Beale & Block, 2011a) (Table I and Figure 3). Antibacterial activities were done against *Escherichia coli* (Gram-negative) of the compounds. The reference drug was amoxicillin as a control positive. All compounds tested for antibacterial activity against E. *coli* showed positive activity but different MIC.

Table I: Result of docking molecule of 2-hydroxybenzohydrazide and derivatives

Compounds	Rerank score (kcal/mol)	Amino acid which holds hydrogen bonds and steric interactions
Methyl salicylate	-64.9327	Ala 1196, Ala 1197 , Tyr 1156, Tyr 1146, Thr 1194
N'-benzylidene-2-hydroxy benzohydrazide	-80.5287	Ala 1196, Ala 1095 , Tyr 1156, Leu 1100, Met 1159
N'-(2-methoxybenzylidene)-2-hydroxybenzohydrazide	-85.9061	Ala 1196, Ala 1095 , Tyr 1156, Leu 1100, Met 1159
N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide	-92.9558	Ala 1196, Ala 1095 , Tyr 1156, Leu 1100, Met 1159, Asn 1155, Asn 1157

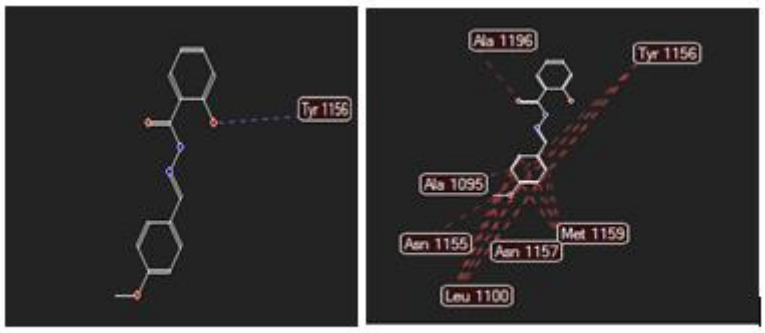

Figure 3: Hydrogen bonding (a) and steric interaction (b) of N'-(4-Methoxy benzylidene)-2-hydroxybenzohydrazide with receptor ENR inhibitor (Pdb.1C14)

Discussion

The results of molecular docking with the ENR inhibitor receptor (Pdb.1C14) showed that the compound N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide had the lowest rank score compared to methyl salicylate. The lower the rerank score, the more stable the bond between the drug and the receptor, so the antibacterial activity is higher (Beale & Block, 2011b; Ali *et al.,* 2012; George *et al.,* 2014). This is consistent with the results of the antibacterial activity test against *Escherichia coli*. N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide has the lowest MIC compared to the others.

The results indicated that N'-(2-methoxybenzylidene)-2-hydroxybenzohydrazide and N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide show activity against E. *coli*. Results of minimal intensive concentration (MIC) of N'-(2-methoxybenzylidene)-2-hydroxybenzohydrazide and N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide is 1000 ppm and 120 ppm. N'-benzylidene-2-hydroxybenzohydrazide has activity against E. *coli* > 1000 ppm. The antibacterial activity of the compound indicated the presence of 4-methoxy and 2-hydroxy groups on the benzene ring and an azo-amide group (-N=N-C=O) (Wang *et al.,* 2011; Asif, 2014; Pangal, Ahmed & Shaikh, 2013). The docking results showed that the 2-hydroxy group on the benzene ring forms a hydrogen bond with the amino acid Tyr 1156 of the receptor. The azo-amide group interacts sterically with the amino acid Ala 1196. The steric interaction of the 4-methoxyphenyl group with the receptor amino acid is greater than that of the 2-methoxyphenyl group (Table I and Figure 3).

Conclusion

It can be concluded that 2-hydroxybenzohydrazide and its derivatives can be synthesised by microwave-irradiation (160-320 Watts of power for 2-8 minutes). The derivatives were N'-benzylidene-2-hydroxybenzohydrazide, N'-(2-methoxybenzylidene)-2-hydroxybenzohydrazide and N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide. The yields were obtained at 68%-81%. The preliminary biological tests indicated that N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide has effective activity against *Escherichia coli*.

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